role in this Ras oligomerization interaction, and discuss the implications for the regulation of up- and downstream effectors.

**509-Pos Board B278**
**The Role of Phosphatidylinositol Phosphates (PIPs) in Poliovirus Replication Complexes Trafficking to Viral Replication Organelles**
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Viruses are intracellular parasites which utilize their host cell's subcellular machinery for their own replication by remodeling of the host's intracellular membranes. Lipids with phosphatidylinositol-4-phosphate (PI4P) head groups have been found to be crucial for the formation of these replication organelles in enteroviruses and flaviviruses. Lipid binding assays show that poliovirus protease 3Cpro specifically binds to PI4P. Using NMR spectroscopy, binding with other phosphatidylinositol phosphates (PIPs) has been detected and specific residues has been identified which exhibits chemical shift changes upon binding with 3Cpro. PI3P, PI4P, and PI5P have been found to exhibit similar chemical shift perturbation patterns upon binding with 3Cpro. Our results indicate that a novel PIP binding site has been identified, with some residues exhibiting long range interactions (or a secondary binding site).

**510-Pos Board B279**
**Surface Electrostatics and Peptide Binding to Lipid Bilayer of Defined Curvature**
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Many proteins are known to have strong binding preferences for curved lipid bilayer surfaces. This property of proteins to sense membrane curvature has a number of implications in cellular processes including endocytosis, exocytosis, and vesicle trafficking as well as pathogenic processes associated with viral infections or protein aggregation disorders. Here we employ small unilamellar vesicles (SUVs) that are sized by extrusion through nanoporous filters as a model of convex membranes with accessible outer surface. For concave structures we developed nanopore-confined tubular lipid bilayers with tunable curvature radii ranging from ca. 40 to 250 nm. The nanotubular bilayers are formed by lipid self-assembly inside cylindrical nanopores of anodic aluminum oxide (AAO). These systems impose essentially no restrictions on the lipid composition and have the inner surface of negative curvature accessible for solute molecules and peptide binding. Using these model structures we show that the surface electrostatic potential of lipid bilayers is affected by the curvature to a rather large degree. For example, for SUVs composed of negatively charged lipids the magnitude of the surface potential increased with bilayer bending from ca. −106 mV for 100 nm SUV to −166 mV for 30 nm SUVs. These measurements were carried out by spin probe EPR method using recently synthesized lipids having pH-reporting nitroxides covalently tethered to the lipid polar head. EPR titration experiments were followed by the measurement of the lipid vesicle electrophoretic mobility and peptide binding. Overall, the data indicate that the bilayer bending affects the local electrostatic potential in a rather large degree providing a likely biophysical mechanism for affecting protein binding to lipid membranes without adjusting lipid composition. Supported by U.S. DOE Contract DE-FG02-02ER15354.

**511-Pos Board B280**
**Mgm1 Alters Membrane Topology and Promotes Local Membrane Bending to Drive Mitochondrial Membrane Fusion**
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Cellular membrane remodeling events such as mitochondrial dynamics, vesicle budding and cell division rely on the large GTPases of the dynamin superfamily. Dynamins have long been characterized as fission molecules; however, how they mediate membrane fusion is largely unknown. Recently, we showed that the mitochondrial dynamin Mgm1 can mediate fusion by first tethering opposing membranes and undergoing nucleotide-dependent structural transition. However, it is still unclear how Mgm1 could act directly on the membrane to drive the fusion of tethered membranes. Here, we show that Mgm1 binding to the membrane could alter membrane topology and promote local membrane bending. By atomic force microscopy, we showed that Mgm1 created roughness and a tubulated structure on a supported lipid bilayer, and by monitoring giant liposomes Mgm1 recruited on and deformed the liposomes. These data suggest Mgm1 lipid interactions and changes in the protein/lipid complexes could apply forces onto the membrane to possibly promote fusion of opposing membrane. Together our data provide a possible mechanism of how Mgm1 mediates mitochondrial fusion and shed light onto how dynamins function as fusion molecules.

**512-Pos Board B281**
**Membrane Remodeling by Curvature-Inducing Proteins**
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A host of membrane-binding proteins are responsible for dynamically remodeling cell membranes during vital cell processes, including endocytosis, exocytosis, cell migration, and morphogenesis. Experiments show that the protein exo70, a component of the exocyst, generates membrane protrusions on cell surfaces. We employ coarse-grained molecular dynamics simulations to validate confocal microscopy results which show that dimerization of exo70 is necessary to induce sufficient curvature for tubular invaginations in synthetic vesicles. These simulations provide mechanistic insight into the molecular details of membrane bending by exo70. Calculation of the local three-dimensional stress tensor reveals the shape and depth of the resulting curvature field and this parameter informs a mesoscale for membrane bending which includes the anisotropy of the induced curvature field. We also probe the role of binding to phosphatidylinositol (4,5)bisphosphate (PIP2), a key membrane constituent which binds many other curvature-inducing proteins including epsin and the Bin-Amphyphsin-Rvs (BAR) domains. PIP2 modulates a number of cellular signaling pathways, and anchors curvature-inducing proteins to the membrane via electrostatic interactions. Our simulations also validate experiments which show that a double mutation of positively charged residues in exo70 disrupts the binding to negatively charged PIP2, abolishing membrane binding and the resulting tubular invaginations. These modeling methods are extended to other curvature-inducing proteins, including the epsin N-terminal homology domain (ENTH), which senses and drives membrane curvature during clathrin-mediated endocytosis. Our simulations provide a link between detailed all-atom simulations, which capture the physics of membrane-protein binding and the role of PIP2, with mesoscopic models for membrane sculpting. These simulations provide molecular details for membrane remodeling by exo70 observed by confocal microscopy in synthetic vesicles, and fluorescence microscopy in live cells.

## Calcium Signaling Proteins

**513-Pos Board B282**
**Site-Specific Measurement of Terbium and Calcium Binding to Calmodulin and Calmodulin Fragments**
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Calcium controls a variety of cellular processes including muscle contraction, cellular motility, and synaptic function. A key player in many of these signaling events is the evolutionarily conserved $Ca^{2+}$ sensor, calmodulin (CaM), a 148 amino acid protein consisting of four EF hand $Ca^{2+}$-binding domains arranged as pairs in two globular domains (N- and C-lobe) connected by a flexible linker. Though extensive work has investigated $Ca^{2+}$ binding to CaM, published $Ca^{2+}$ association constants and cooperativity factors vary by several orders of magnitude. Surprisingly little of this variability is attributable to differences in experimental conditions. We have determined through simulations that macroscopic total binding curves are fundamentally insufficient to constrain a complete, quantitative model of $Ca^{2+}$ binding to CaM. To improve estimates of $Ca^{2+}$ affinity and cooperativity in binding to CaM we have conducted site-specific binding measurements at each binding site. To detect metal occupancy at individual sites, terbium ($Tb^{3+}$) ions, which have been shown to effectively substitute for $Ca^{2+}$ in activating CaM, are sensitized via energy transfer from a minimally invasive tryptophan residue inserted in each EF hand. Through competition experiments, both $Ca^{2+}$ and $Tb^{3+}$ association constants can be determined. We have obtained site-specific $Tb^{3+}$ binding isotherms at all four sites in full CaM and measurements of $Tb^{3+}$ binding and $Ca^{2+}$ competition in an isolated, N-lobe CaM fragment. These data suggest distinct $Ca^{2+}$ and $Tb^{3+}$ affinities at each of the sites in the N-lobe CaM fragment and distinct $Tb^{3+}$ affinities at all four sites in full CaM. The additional information obtained through site-specific binding measurements enhances estimates of $Ca^{2+}$ affinity and cooperativity in binding to CaM. This technique is powerful in its applicability to a variety of EF-hand containing calcium-sensing proteins in isolation or in complex with signaling partners.